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Attachment A

Roitt et al., Immunology, 1.3-2.15

IMMUNOLOGY

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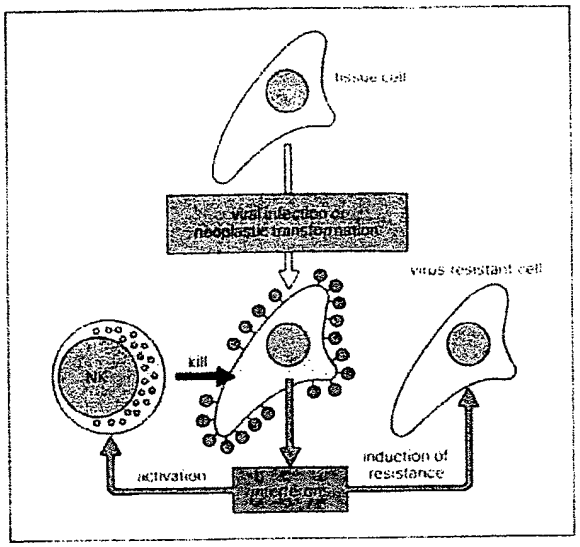

Fig. 1.7 Interferon and NK cells. When a cell becomes infected by virus, or transforms into a cancerous cell its surface molecules are altered. These alterations can sometimes be recognized by natural killer (NK) cells which engage the cell and kill it. Virally-infected cells produce interferons which can signal to neighbouring tissue cells and put them into a state capable of resisting viral replication, so preventing virus spread. Additionally, interferons can activate NK cells and enhance their cytotoxic action.

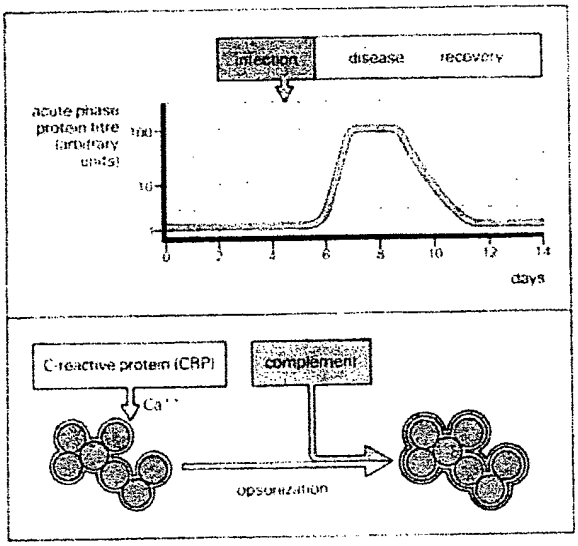

Fig. 1.8 Acute phase proteins. Acute phase proteins (here exemplified by C-reactive protein) are serum proteins which increase rapidly in concentration (up to 100 fold) following infection (graph). They are important in the innate immunity to infection. C-reactive protein (CRP) recognizes and binds, in a Ca^{++} dependent fashion, to molecular groups found on a wide variety of bacteria and fungi. In particular it binds the phosphorylcholine moiety of pneumococci. The CRP acts as an opsonin and also activates complement with all the associated sequelae.

A further group of complement components causes direct lysis of the cell membranes of bacteria by the 'lytic pathway' (Fig. 1.9). Although the various molecules of the innate immune system have been described separately, *in vivo* they act in concert. For example, the destruction of bacterial cell walls by lysozyme facilitates an attack on the cell membrane by the lytic pathway complement components. As will become evident later the complement system performs a number of functions in addition to its action in opsonization and lysis of microorganisms. These can be summarized as the control of inflammation.

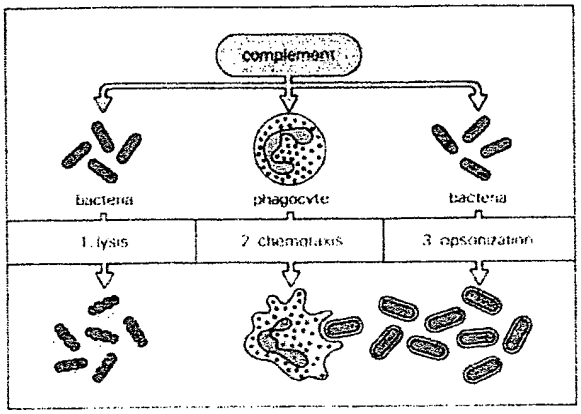

Fig. 1.9 Complement functions. The complement system has an intrinsic ability to lyse the cell membranes of many bacterial species (1). Complement products released in this reaction attract phagocytes to the site of the reaction – chemotaxis (2). Once they arrive at the site of reaction other complement components coating the bacterial surface allow the phagocyte to recognize the bacteria and facilitate bacterial phagocytosis — opsonization (3). These are all functions of the innate immune system, although the reactions can also be triggered by the adaptive immune system.

INFLAMMATION

Inflammation is the body's reaction to an injury such as an invasion by an infectious agent. In just the same way as it is necessary to increase the blood supply to active muscles during exercise to provide glucose and oxygen so it is also necessary to direct elements of the immune system into sites of infection. Three major things occur during this response namely:

1. An increased blood supply to the infected area,
2. Increased capillary permeability caused by retraction of the endothelial cells. This permits larger molecules to traverse the endothelium than would ordinarily be capable of doing so and thus allows the soluble mediators of immunity to reach the site of infection,
3. Leucocytes, particularly neutrophil polymorphs and to a lesser extent macrophages, migrate out of the capillaries and into the surrounding tissue. Once in the tissue they migrate towards the site of infection by a process known as chemotaxis. These three events manifest themselves as inflammation.

Chemotaxis

Chemotaxis is the process by which phagocytes are attracted to sites of inflammation (Fig. 1.10). It can be demonstrated *in vitro* that phagocytes will actively migrate up a concentration gradient of certain (chemotactic) molecules. Particularly active is C5a, a fragment of one of the complement components. When purified C5a is applied to the base of an ulcer *in vivo* neutrophil polymorphs can be seen sticking to the endothelium of the nearby capillaries shortly afterwards. Initially this occurs on the side of the capillary nearest the point of application but as the C5a diffuses further the neutrophils stick to all sides of the endothelium before traversing the endothelium, crossing the basement membrane and migrating up the gradient of the chemotactic molecule. Adherence and diapedesis of leucocytes is illustrated in figures 1.11 and 1.12. Both neutrophil polymorphs and macrophages are attracted by C5a but neutrophils are the predominant cell in sites of acute inflammation reflecting their numerical preponderance in the blood.

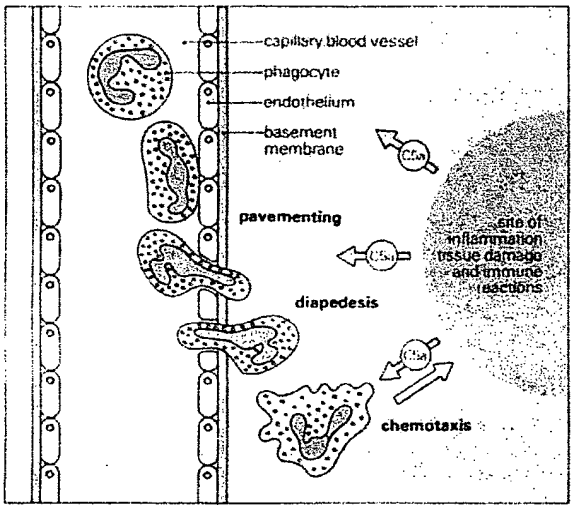

Fig. 1.10 Chemotaxis. At a site of inflammation tissue damage and complement activation by the infectious agent cause the release of chemotactic peptides (eg. C5a, a fragment of one of the complement components, which is one of the most important chemotactic peptides). These peptides diffuse to the adjoining capillaries causing passing phagocytes to adhere to the endothelium (pavementing). The phagocytes insert pseudopods between the endothelial cells and dissolve the basement membrane (diapedesis). They then pass out of the blood vessel and move up the concentration gradient of the chemotactic peptides towards the site of inflammation.

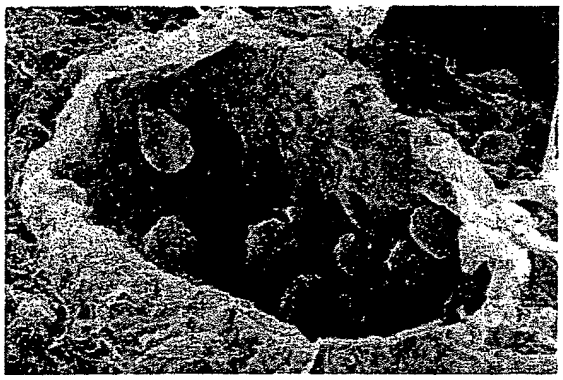

Fig. 1.12 Scanning electron micrograph showing leucocytes adhering to the wall of a venule in inflamed tissue. ×16,000. Courtesy of Professor M. J. Karnovsky.

Phagocytosis

Once they have arrived at a site of inflammation the phagocytes have to recognize the infectious agent. They have receptors on their surface which allow them to attach non-specifically to a variety of microorganisms, but the attachment is greatly enhanced if the microorganism has been opsonized by the C3b component of complement. Complement activation at the site of infection causes C3b to be deposited on the infectious agent and since both neutrophils and macrophages have receptors which specifically bind to C3b this allows the phagocytes to recognize their targets (Fig. 1.13). The importance of complement opsonization can be seen in those very rare patients who are genetically deficient in complement component C3. These patients suffer from recurrent bacterial infections and septicaemia.

Fig. 1.11 Electron micrographs showing the three phases of diapedesis. The first micrograph shows a leucocyte adhering to the capillary endothelium (left) before it penetrates the endothelium (middle). The third micrograph illustrates a leucocyte which has traversed the endothelium (right). X4000. Courtesy of Dr. I. Jovis.

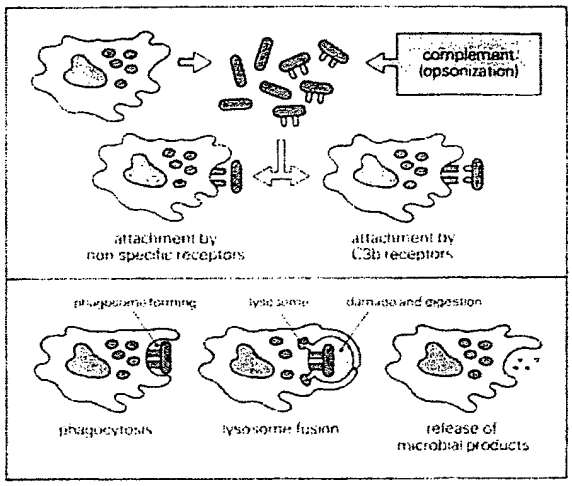

Fig. 1.13 Phagocytosis. Phagocytes arrive at a site of inflammation by chemotaxis. They may then attach to microorganisms via their non-specific cell surface receptors, or if the organism is opsonized with a fragment of the third complement component (C3b) through activation of the complement system, attachment will be through the cell surface receptors for C3b. If the membrane now becomes activated by the attached infectious agent, it is taken into a phagosome by pseudopods which extend around it. Once inside, lysosomes fuse with the phagosome forming a phagolysosome and the infectious agent is killed by a battery of microbicidal mechanisms. Undigested microbial products may be released to the outside.

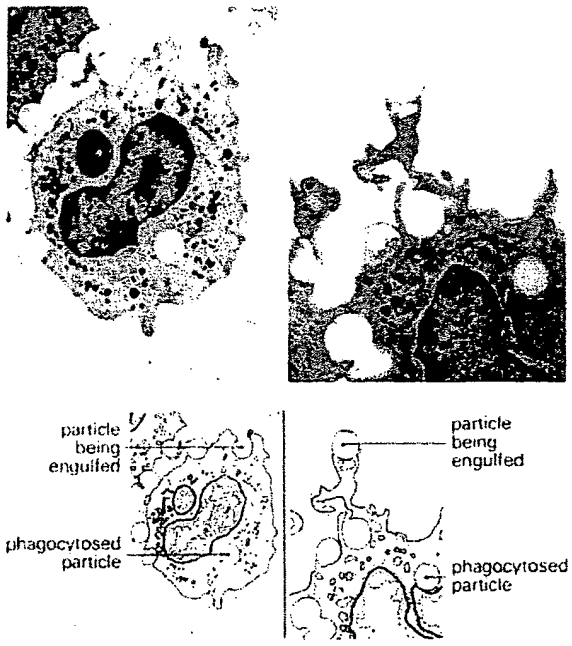

Fig. 1.14 Electron micrographic study of phagocytosis. These two micrographs show human phagocytes engulfing latex particles. ×3000 (left), ×4500 (right). Courtesy of Professor C. H. W. Horne.

After attachment the phagocytes proceed to engulf the microorganism by extending pseudopods around it. These fuse and the microorganism is internalized in a phagosome (Fig. 1.14). Lysosomes fuse with the phagosome and destroy the trapped microorganism. The mechanisms involved are described more fully in 'Immunity to Viruses, Bacteria and Fungi' and 'Immunity to Protozoa and Worms'.

ANTIBODY — A FLEXIBLE ADAPTOR

Problems arise when the phagocytes are unable to recognize the infectious agent either because they lack a suitable receptor for it or because the microorganism does not activate complement and so cannot become attached to the phagocyte via the C3b receptor. Ideally, what is needed is a flexible adaptor that can attach at one end to the microorganism and at the other to the phagocyte. In answer to this requirement molecules known as antibodies have evolved and these are fully described in 'Antibody Structure and Function'. Antibodies are a class of molecules produced by B lymphocytes of the adaptive immune system which act as flexible adaptors between the infectious agents and phagocytes (Fig. 1.15).

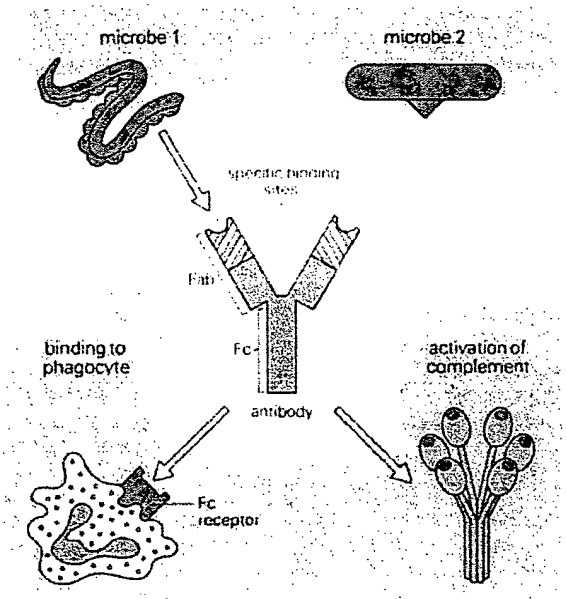

Fig. 1.15 Antibody — a flexible adaptor. When a microorganism lacks the inherent ability to activate complement or phagocytes, the body provides a class of flexible adaptor molecules with a series of different shapes which can attach to the surface of different microbes. These flexible adaptor molecules are, of course, antibodies and the body can make several million different antibodies able to recognize a wide variety of infectious agents. Thus the antibody illustrated binds microbe 1, but not microbe 2, by its 'antigen binding portion' (Fab) while the 'Fc portion' (which may activate complement) binds to Fc receptors on host tissue cells, particularly phagocytes.

THE POLYMORPHONUCLEAR GRANULOCYTES (POLYMORPHS)

Granulocytes are produced in the bone marrow at a rate of eighty million per minute and are short-lived (2-3 days) relative to monocyte/macrophages which may live for months or years. Granulocytes represent about 60 to 70% of the total normal blood leucocytes but are also found in extravascular sites. Polymorphs are able to adhere to and penetrate the endothelial cells lining the blood vessels. As the name suggests, the mature forms usually contain a multi-lobed nucleus and many granules. They are classified into neutrophils, eosinophils and basophils on the basis of the staining reaction of their granules by histological dyes.

Although these cells do not show any specificity for antigens they play an important role in acute inflammation and, together with antibodies and complement, in protection against microorganisms. The predominant role of polymorphs is phagocytosis and their importance in protection is emphasized by the great increase in susceptibility to infections found in individuals with low numbers of circulating polymorphs.

Neutrophils

Neutrophils represent over 90% of the circulating granulocytes and are 10-20μ in diameter (Fig. 2.28). They possess two main types of granules. The primary (azurophilic) granules (lysosomes) contain acid hydrolases, myeloperoxidase and muraminidase (lysozyme) whilst the secondary or specific granules contain lactoferrin in addition to lysozyme. These granules can be seen at the ultrastructural level (Fig. 2.29). Ingested organisms are contained within vacuoles termed phagosomes which fuse with the enzyme-containing granules to form the phagolysosomes (Fig. 2.30).

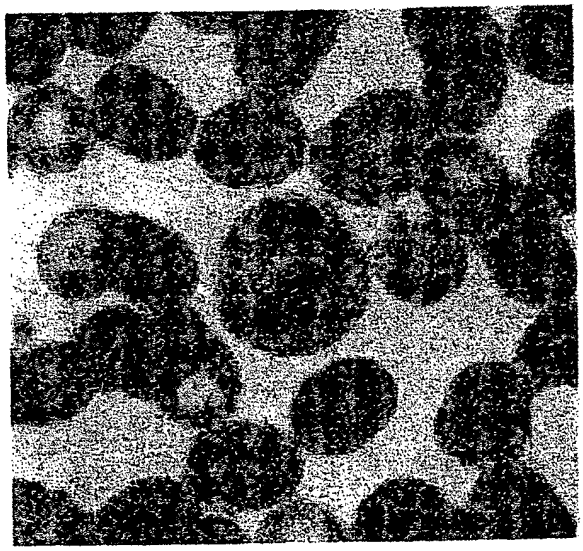

Fig. 2.28 Morphology of the neutrophil. This blood smear shows a neutrophil with its characteristic polymorphonuclear shape and neutrophilic cytoplasm. Giemsa stain, ×4500

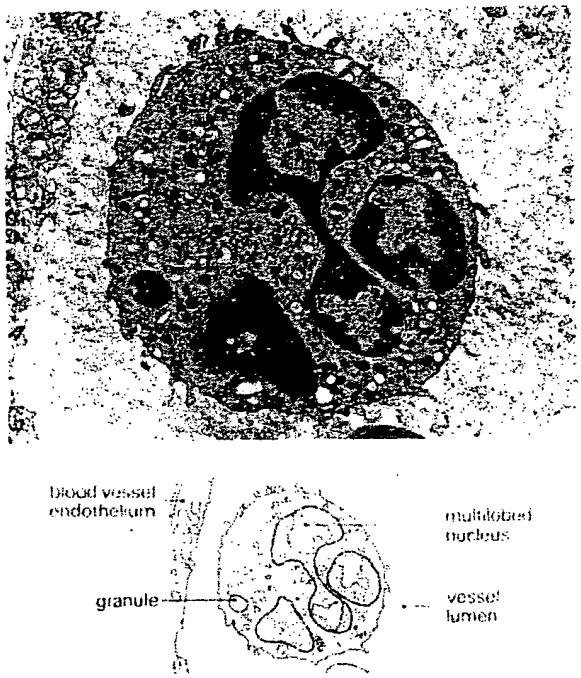

Fig. 2.29 Electron micrograph showing the ultrastructure of the neutrophil. This mouse neutrophil lies within a skin blood vessel. The neutrophil cytoplasm contains primary and secondary granules of different electron opacity. ×10,000. Courtesy of Dr. D. McLaren

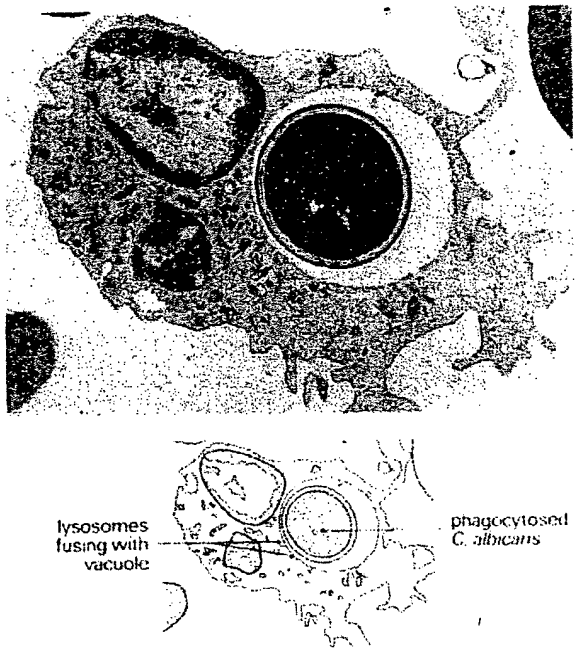

Fig. 2.30 Electron micrograph showing a neutrophil containing phagocytosed *Candida albicans*. Two lysosomal granules may be seen fusing with the vacuole containing the organism. ×7000. Courtesy of Dr. H. Valdimarsson.

Eosinophils

Eosinophils comprise 2-5% of blood leucocytes in healthy, non-allergic individuals (Fig. 2.31). Like neutrophils they do appear to be capable of phagocytosing and killing ingested microorganisms, although it is not their primary function. The granules in mature eosinophils are membrane-bound organelles with a 'crystalloid' or 'core' differing in electron opacity from the surrounding matrix (Fig. 2.32). Human blood eosinophils usually have only a bilobed nucleus and many cytoplasmic vesicles. They possess many ribosomes, mitochondria, and microtubules, suggesting that they are metabolically active.

Eosinophils (as well as basophils and mast cells described below) can be triggered to degranulate by appropriate stimuli. Degranulation involves fusion of the intracellular granules with the plasma membrane. The contents are released to the outside of the cell. This type of reaction is the only way that these cells can use their 'granule armament' against large targets which cannot be phagocytosed. Eosinophils are thought to play a specialized role in the immunity to helminth infections using this mechanism (see 'Immunity to Protozoa and Worms').

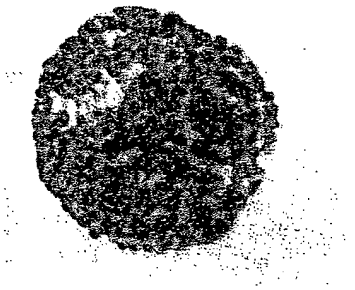

Fig. 2.31 Morphology of the eosinophil. This blood smear enriched for granulocytes shows an eosinophil with its multilobed nucleus and heavily-stained cytoplasmic granules. Leishman stain, ×5000.

Eosinophils are attracted by products released from T cells, mast cells and basophils (Eosinophil Chemotactic Factor of Anaphylaxis, ECF-A). They bind schistosomulae coated with IgG antibody, degranulate, and release a toxic protein ('major basic protein'). Eosinophils release histaminase and aryl sulphatase, which inactivate the mast cell products histamine and Slow Reactive Substance of Anaphylaxis (SRS-A) respectively. The net effect of these factors is to dampen down the inflammatory response and reduce granulocyte migration into the site of invasion.

Basophils and Mast Cells

Basophils are found in very small numbers in the circulation (less than 0.2% of the leucocytes) and are characterized by deep violet blue granules (Fig. 2.33). The mast cell is often indistinguishable from the basophil in a number of its properties and although they are both of bone marrow origin its relationship to the basophil is not completely clear.

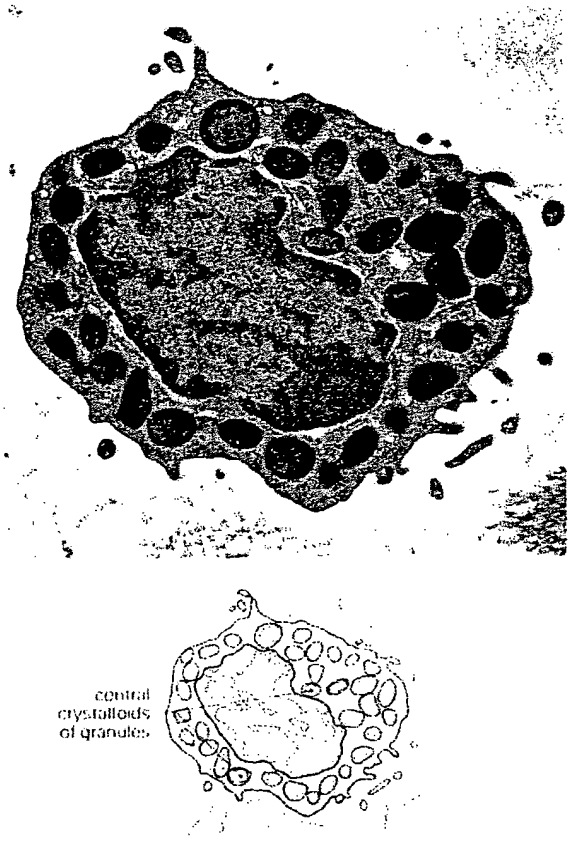

Fig. 2.32 Electron micrograph showing the ultrastructure of a guinea pig eosinophil. The mature eosinophil contains granules with central crystalloids. × 11,500. Courtesy of Dr. D. McLaren.

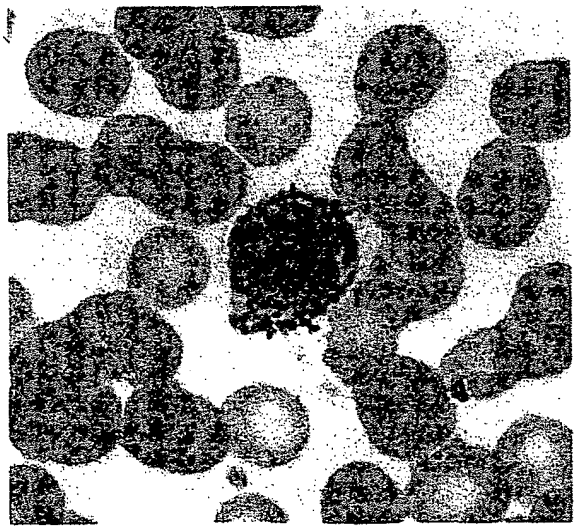

Fig. 2.33 Morphology of the basophil. This blood smear shows a typical basophil with its deep violet-blue granules. Wright's stain, ×4500.

Fig. 2.34 Histological appearance of human (gut) connective tissue mast cells. This micrograph shows the dark blue cytoplasm with brownish granules. Alcian blue and Safranin. ×2500.

Mast cells are found associated with mucosal epithelial cells where they appear to be dependent on T cells for their proliferation. In addition, they are commonly found in the connective tissue where they are T cell independent. Under light microscopy, they can be visualized with Alcian blue (Fig. 2.34).

Mature blood basophils have randomly distributed granules surrounded by, and containing membranes (Fig. 2.35). These granules in both basophils and mast cells

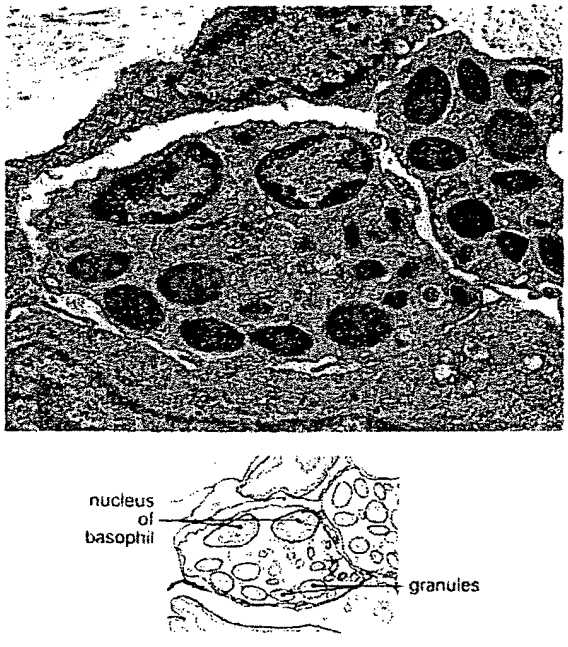

Fig. 2.35 Electron micrographs showing the ultrastructure of the basophil. Basophils in guinea pig skin showing the characteristic randomly distributed granules. ×10,000. Courtesy of Dr. D. McLaren.

contain heparin, SRS-A and ECF-A and these are released on degranulation initiated by the appropriate stimulus. This is usually an allergen which cross-links specific IgE molecules bound to the surface of the mast cell or basophil via Fc receptors for IgE (Fig. 2.36). Pharmacological mediators released following degranulation cause the adverse symptoms of allergy but, on the positive side, they may also play a role in immunity against parasites. Granulocyte markers are summarized in figure 2.37.

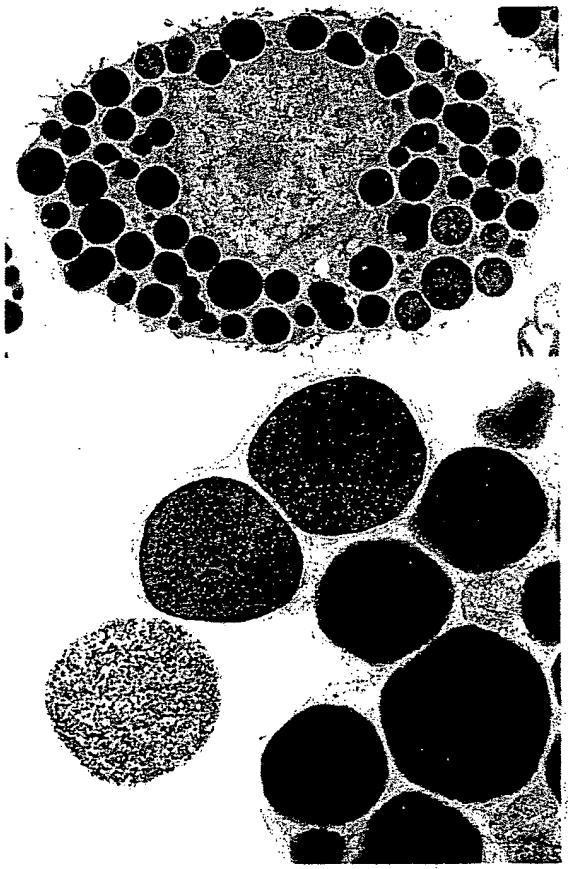

Fig. 2.36 Electron micrographs of rat peritoneal mast cells. These show the undegranulated cell with its electron-dense granules (upper, ×6000) and a granule in the process of exocytosis (lower, ×30,000). Courtesy of Dr. T. S. C. Orr.

Platelets

The final myeloid cell to be considered here is the blood platelet. In addition to their role in blood clotting, platelets are also involved in the immune response, especially in inflammation. They possess class I MHC products and receptors for both IgG and IgE. Platelets are derived from large megakaryocytes in the bone marrow and are seen to contain granules at the ultrastructural level (Fig. 2.38). Following endothelial injury, platelets adhere to and aggregate at the endothelial surface releasing permeability-increasing substances and factors responsible for activating complement components to attract leucocytes.

					CR1	CR3	peroxidase	acid phosphatase	alkaline phosphatase
neutrophil	−	+	+	+	+	+	+	+	+
eosinophil	+	+	+?	+	+	+	+	+	
basophil	+	+	+	+	+	+	+		
mast cell	+	+	+	+	+	+		+	+

Fig. 2.37 Summary of the surface markers on mature human granulocytes. All cells possess Fc receptors for IgG (FcγR). Only basophils and mast cells have high affinity receptors for IgE ($Fc_\epsilon R$); eosinophils have low affinity receptors. All cells carry receptors for complement components: receptors for C3a and C5a are important for chemotaxis; and CR1 and CR3 are involved in adherence and phagocytosis. The granules in different cell types vary qualitatively in their enzyme content.

Fig. 2.38 Electron micrograph showing the platelet ultrastructure. The cytoplasmic organelles, including granules and mitochondria, are randomly dispersed. ×20,000. Courtesy of Dr. J. G. White.

SUMMARY

There are several types of cells involved in the immune response. Some cells possess the basic primitive function of phagocytosis and intracellular killing. This can be augmented by antibodies and complement components. Other cells, which are not phagocytic, present antigen to the more advanced lymphocytes. T and B lymphocytes with specialized functions are able to respond, specifically through complex cellular interactions, to defined antigenic determinants. Unlike the primitive immune system of lower animals, the vertebrate immune system exhibits memory. That is to say that a secondary antigenic challenge usually produces a greater and more effective response than the primary challenge. The function of memory, as well as specificity, is dependent on the lymphocytes.